

PMRA Submission Number: 2008-0431

PMRA Document ID: 1608338

EPA MRID Number: 47445903

**DATA EVALUATION RECORD**  
**HONEY BEE - ACUTE CONTACT & ORAL LC<sub>50</sub> TEST**  
**§141-1 & Nonguideline**

<b>Data Requirement:</b>	PMRA Data Code	9.2.4.1 and 9.2.4.2
	EPA DP Barcode	DP354470
	OECD Data Point	IIA 8.7.2
	EPA MRID	47445903
	EPA Guideline	OPPTS 850.3020
	OECD Guideline	OECD 214

1. **CHEMICAL:** Saflufenacil PC Code No.: 118203

2. **TEST MATERIAL:** BAS 800 01 H Purity: 68.8% (analyzed)

3. **CITATION**

Authors: Kling, A.

Title: Assessment of Side Effects of BAS 800 01 H to the Honey Bee, *Apis mellifera* L. in the Laboratory

Study Completion Date: February 11, 2008

Laboratory: eurofins-GAB GmbH  
Eutingen, Str. 24, D-75223 Niefern-Öschelbronn  
Germany

Sponsor: BASF Corporation  
Agricultural Products Division  
Research Triangle Park, North Carolina 27709

Laboratory Report ID: 317342

MRID No.: 474459-03

DP Barcode: D354470

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

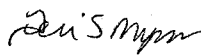
**Signature:**



**Date:** 07/21/08

**APPROVED BY:** Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

**Signature:**




**Date:** 07/23/08



2083881

5. **APPROVED BY:** Primary Reviewer: Anita Pease, Senior Biologist, U.S. EPA

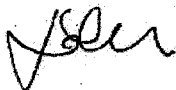
Signature:

  
6/9/09

Date: 06/09/09

Secondary Reviewer: Janine Glaser, HC-PMRA-EAD

Signature:



Date: 06/09/09

Secondary Reviewer: Farzad Jahromi, DEWHA-APVMA

Signature:



Date: 06/09/09

6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to honey bees via oral and contact exposure routes. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. **EXECUTIVE SUMMARY:** The honey bee, *Apis mellifera* L., was exposed to BAS 800 01 H (68.8% Saflufenacil) for 48 hours in oral and the contact tests. The oral and contact nominal concentrations were 0 (negative control) and 100 µg a.i./bee. The actual intake concentration of BAS 800 01 H in the oral toxicity test was 120.90 µg a.i./bee. By 48 hours in the oral test, mortality was 0% in the control and 2.0% in the 120.90 µg a.i./bee treatment group; all surviving bees appeared normal and healthy throughout the oral test. By 48 hours in the contact test, mortality was 0% in the control and 2.0% in 100 µg a.i./bee treatment group; all surviving bees appeared normal and healthy throughout the test. **The LC<sub>50</sub> value for the oral test was >120.90 µg a.i./bee. The LD<sub>50</sub> value for the contact test was >100 µg a.i./bee. As a result, BAS 800 01 H (68.8% Saflufenacil) is categorized as practically non-toxic to honey bees on an acute contact basis.** The NOAELs for the oral and contact tests were 120.90 and 100 µg a.i./bee, respectively.

The contact toxicity test is classified as **ACCEPTABLE** to U.S. EPA and as **FULLY RELIABLE** to PMRA and APVMA. The oral toxicity test is classified as **SUPPLEMENTAL** to U.S. EPA because it does not fulfill any current U.S. EPA data requirement; the oral toxicity test is classified as **FULLY RELIABLE** to PMRA and APVMA.

#### **8. STUDY PARAMETERS:**

<b>Scientific Name of Test Organism:</b>	<i>Apis mellifera</i> L.
<b>Age of Test Organism at Test Initiation:</b>	Young adult worker bees
<b>Type of Concentrations:</b>	Nominal (contact) and actual uptake (oral)
<b>Definitive Test Duration:</b>	48 hours for both tests

#### **9. CONCLUSIONS:**

##### **Reported Statistical Results - Oral Test:**

LC <sub>50</sub> : >120.90 µg ai/bee	95% C.I.: N/A
NOAEL: 120.90 µg ai/bee	Probit Slope: N/A
LOAEL: >120.90 µg ai/bee	

##### **Reported Statistical Results - Contact Test:**

LD <sub>50</sub> : >100 µg ai/bee	95% C.I.: N/A
NOAEL: 100 µg ai/bee	Probit Slope: N/A
LOAEL: >100 µg ai/bee	

#### **10. ADEQUACY OF THE STUDY:**

**A. Classification:** The contact toxicity test is classified as **ACCEPTABLE** to U.S. EPA and as **FULLY RELIABLE** to PMRA and APVMA. The oral toxicity test is classified as **SUPPLEMENTAL** to U.S. EPA because it does not fulfill any current U.S. EPA data requirement; the oral toxicity test is classified as **FULLY RELIABLE** to PMRA and APVMA.

**B. Rationale:** Not applicable

**C. Repairability:** Not applicable

**11. GUIDELINE DEVIATIONS:** This study was conducted following guidelines outlined in OECD Guideline No. 213- Honey bees, acute oral toxicity test; and OECD Guideline No. 217- Honey bees, acute contact toxicity test. There is currently no OPPTS guidance for acute honey bee oral toxicity tests. The following guideline deviation in the honey bee contact toxicity test (OPPTS 850.3020) was noted:

The temperature in this study ranged slightly lower (24°C) than the minimum recommended by the OPPTS 850.3020 guideline (25°C).

This deviation does not affect the acceptability of this study.

**12. SUBMISSION PURPOSE:** This study was submitted to provide data on the effects on honey bees following acute contact and oral exposure to BAS 800 01 H (Saflufenacil) in the laboratory for the purpose of new chemical registration.

**13. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b>Species:</b> Species of concern ( <i>Apis mellifera</i> , <i>Megachile rotundata</i> , or <i>Nomia melanderi</i> )	<i>Apis mellifera</i> L.
<b>Age at beginning of test:</b>	Young, adult worker bees
<b>Supplier:</b>	Bees were obtained from a healthy colony, maintained by Mr. Carlos Feuerriegel, Ciudad Jardin 54, 44620 Ayora, Spain.
<b>All bees from the same source?</b>	Yes

**B. Test System**

Guideline Criteria	Reported Information
--------------------	----------------------

Guideline Criteria	Reported Information
Cage size adequate?	Cages were made of high grade steel (10 cm wide x 5.5 cm deep x 8.5 cm high) with the front sides equipped with a transparent pane so that bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test insects. The test cages were lined with filter paper.
Lighting:	Bees were kept under constant darkness, except for observations, during which test cages were illuminated with neon light.
Temperature:	24.0-25.0°C
Relative humidity:	53-54%

### C. Test Design

Guideline Criteria	Reported Information
Range finding test?	N/A; no range-finding data were provided. However, this study was conducted as a limit test with a single nominal concentration of 100 µg a.i./bee.
Reference toxicant test?	Perfekthion (dimethoate, 595.9 g/L)  <u>Oral test:</u> 0.08, 0.12, 0.18 and 0.30 µg a.i./bee <u>Contact test:</u> 0.10, 0.14, 0.26 and 0.36 µg a.i./bee

Guideline Criteria	Reported Information
<b>Method of administration:</b>	<p><u>Oral test:</u> 250 µL of appropriately dosed feeding solution was provided to each cage; bees were starved for 2 hours prior to introduction of food</p> <p><u>Contact test:</u> Bees were anaesthetized and individually treated with a 2 µL droplet placed on the dorsal thorax of each bee using a microapplicator</p>
<b>Nominal doses:</b>	<p><u>Oral test:</u> 100 µg a.i./bee</p> <p><u>Contact test:</u> 100 µg a.i./bee</p>
<b>Controls:</b> Negative control and/or diluent/solvent control	<p><u>Oral test:</u> untreated 50% aqueous sucrose solution</p> <p><u>Contact test:</u> Mineral water</p>
<b>Number of colonies per group:</b>	<p><u>Oral test:</u> 5 replicates, with 10 bees per rep</p> <p><u>Contact test:</u> 5 replicates, with 10 bees per rep</p>
<b>Solvent:</b> The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	<p><u>Oral test:</u> N/A; a solvent was not used</p> <p><u>Contact test:</u> N/A; a solvent was not used</p>
<b>Feeding:</b>	<p><u>Oral test:</u> Treated feed was provided for approximately 6 hours, after which feeders were replaced with untreated 50% sucrose solution</p> <p><u>Contact test:</u> an untreated 50% sucrose solution was provided <i>ad libitum</i></p>

PMRA Submission Number: 2008-0431  
PMRA Document ID: 1608338

EPA MRID Number: 47445903

Guideline Criteria	Reported Information
Observations period:	<u>Oral test</u> : 4, 24 and 48 hours <u>Contact test</u> : 4, 24 and 48 hours

**14. REPORTED RESULTS:**

<b>Guideline Criteria</b>	<b>Reported Information</b>
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the most recent edition of the Principles of Good Laboratory Practice (GLP), Chemikaliengesetz, attachment 1, Germany; and the OECD Principles of Good Laboratory Practice
<b>Control performance:</b>	<u>Oral test:</u> 0% mortality <u>Contact test:</u> 0% mortality
<b>Raw data included:</b>	Yes
<b>Signs of toxicity (if any) were described?</b>	No sub-lethal effects were observed in the control or Saflufenacil treatment group in either test.



**Mortality - Oral Test**

Dosage µg a.i./bee (actual intake)	No. of Bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance			
Control	50	0.0	0.0
120.90	50	2.0	2.0
Perfekthion (Dimethoate)			
0.10	50	10.0	14.0
0.14	50	44.0	50.0
0.19	50	74.0	88.0
0.27	50	92.0	92.0

Observations: After 48 hours, mortality was 0 and 2.0% in the control and 120.90 µg a.i./bee treatment group, respectively. All surviving bees were normal and healthy throughout the test, yielding NOAEC and LC<sub>50</sub> values of 120.90 and >120.90 µg a.i./bee, respectively.

The 48-hour oral LC<sub>50</sub> (and 95% C.I.) for the toxic standard was 0.14 (0.13-0.15) µg a.i./bee.

**Mortality - Contact Test**

Dosage µg a.i./bee	No. of Bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance			
Negative Control	50	0.0	0.0
100	50	2.0	2.0
Toxic Standard			
0.10	50	14.0	16.0
0.14	50	22.0	26.0
0.26	50	50.0	60.0
0.36	50	88.0	92.0

Observations: After 48 hours, mortality was 0 and 2.0% in the control and 100 µg a.i./bee treatment group, respectively. All surviving bees were normal and healthy throughout the test, yielding NOAEC and LD<sub>50</sub> values of 100 and >100 µg a.i./bee, respectively.

The 48-hour contact LD<sub>50</sub> (and 95% C.I.) for the toxic standard was 0.20 (0.17-0.22) µg a.i./bee.

Statistical method: The average mortality of the five replicates per concentration was calculated after correction for control mortality according to the formula of Schneider-Orelli (1947).

The LD<sub>50</sub> values with 95% confidence intervals were calculated by a means of a probit analysis using the statistic program SAS V 9.1.3, Service Pack 4 (Ed. 2002-2003).

**Reported Statistical Results - Oral Test:**

LC<sub>50</sub>: >120.90 µg a.i./bee  
NOAEL: 120.90 µg a.i./bee

95% C.I.: N/A  
Probit Slope: N/A

LOAEL: >120.90 µg a.i./bee

**Reported Statistical Results - Contact Test:**

LD <sub>50</sub> : >100 µg a.i./bee	95% C.I.: N/A
NOAEL: 100 µg a.i./bee	Probit Slope: N/A
LOAEL: >100 µg a.i./bee	

**15. VERIFICATION OF STATISTICAL RESULTS:**

Statistical method: Because mortality did not exceed 2.0% in the treatment group in either test, no statistical analyses were required. The reviewer visually determined the toxicity values based on the nominal contact concentrations and the actual oral uptake concentrations.

**Results - Oral Test:**

LC <sub>50</sub> : >120.90 µg a.i./bee	95% C.I.: N/A
NOAEL: 120.90 µg a.i./bee	Probit Slope: N/A
LOAEL: >120.90 µg a.i./bee	

**Results - Contact Test:**

LD <sub>50</sub> : >100 µg a.i./bee	95% C.I.: N/A
NOAEL: 100 µg a.i./bee	Probit Slope: N/A
LOAEL: >100 µg a.i./bee	

**16. REVIEWER'S COMMENTS:**

The reviewer's results were identical to those of the study author.

The study author reported that the results are considered to be valid because the mean mortality of the control in the oral and contact toxicity test was ≤10%, the LD<sub>50</sub>/24h of the reference item in the oral toxicity test was within the range of 0.10-0.35 µg ai/bee, and the LD<sub>50</sub>/24h of the reference item in the contact toxicity test was within the range of 0.10-0.30 µg ai/bee.

Stock solution for the contact toxicity test was prepared by dissolving 1429 mg BAS 800 01 H to a final volume of 20 mL with mineral water. For the oral toxicity test, an

equivalent mass of BAS 800 01 H was dissolved in 20 mL mineral water; 2 mL of this solution was diluted with a 50% aqueous sucrose solution to a final volume of 20 mL. The in-life portion of the toxicity test was conducted from December 11 to December 13, 2007.

## **17. REFERENCES:**

Abbott, W.S. 1925: A method of computing the effectiveness of an Insecticide. J. of Econ. Entomol. 18; 265-267.

ICPBR (2000) Hazards of pesticides to bees. 7<sup>th</sup> International Symposium of the ICPBR Bee Protection Group, Avignon (France), 07-09 September 1999; Les Colloques d'INRA.

OECD Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17, Organization for Economic Cooperation and Development (OECD), Environmental Health and Safety Publications, Paris 1998.

OECD, 1998 Guideline for the testing of chemicals; Honey bees; acute oral toxicity test; 213.

OECD, 1998 Guideline for the testing of chemicals; Honey bees; acute contact toxicity test, 214.

SAS Institute Inc (Ed.), 2002-2003. SAS Version 9.1.3, service pack 4, TS Level 1M3, WIN\_PRO platform; Cary, NC 27513, USA.

Schneider-Orelli, O. (1947). Entomologisches Praktikum. Aarau, 2. Auflage.